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The impact of hepatitis E in the liver transplant setting

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Summary

Hepatitis E virus (HEV) infection has been identified as a cause of graft hepatitis in liver transplant recipients. The true frequency and clinical importance of HEV infections after liver transplantations is a matter of debate. It is proposed that consumption of HEV-contaminated undercooked meat is a main source for HEV infections in developed countries – which might also account for some hepatitis E cases after organ transplantation. However, HEV is also transmitted by transfusion of blood products, likely representing a previously underestimated risk particularly for patients in the transplant setting. HEV infection can take chronic courses in immunocompromised individuals, associated in some cases with rapid progression to cirrhosis within 1–2 years of infection. Diagnosis in transplanted patients is based on HEV RNA testing as antibody assays are not sensitive enough. Selection of immunosuppressive drugs is important as different compounds may influence viral replication and the course of liver disease. Ribavirin has antiviral activity against HEV and should be administered for at least three months in chronically infected individuals; however, treatment failure may occur. HEV infections have also been linked to a variety of extrahepatic manifestations both during and after resolution of infection.

In this review we summarize the emerging data on hepatitis E with a particular focus on the importance of HEV infections for liver transplant recipients.

Introduction

Hepatitis E is caused by infection with the hepatitis E virus (HEV). An infectious agent, leading to acute hepatitis that differed from HAV and HBV, was already suspected in the 1970s. In 1983, Balayan and colleagues showed that oral administration of pooled stool extracts from patients with non-A/non-B hepatitis led to acute hepatitis in a human volunteer in whom virus-like particles were identified in stool samples [1]. Hepatitis E was initially recognized only as an acute self-limited liver disease, which very rarely progressed to acute liver failure. Severe courses were most often observed in pregnant women [2,3] and individuals with chronic liver diseases [4–7]. HEV infections were reported mainly from endemic countries such as the Indian subcontinent, South-East Asia and Sub-Saharan Africa. During the last 3 decades large scale outbreaks of hepatitis E were reported [8–11], even continuing until recently when many hepatitis E cases were reported in refugee camps in South Sudan [12].

For more than 25 years HEV infection was not considered a major clinical problem in developed countries including Europe and the United States. The description of chronic courses of HEV infections in solid organ transplant recipients in France in 2008 [13] increased the awareness for a potentially largely underestimated disease. Since then, persistent HEV infections were described in different cohorts of immunocompromised patients [9,14] but also beyond organ transplantation [15–19]. Subsequently, mechanisms leading to persistent infection were explored in more detail and antiviral therapies were tested in large case series. Importantly, HEV infections seem to take very rapid and aggressive courses in many patients receiving immunosuppressive medications and HEV has been linked with end-stage liver disease and even liver-associated mortality. HEV infections seem to be much more common in Europe than previously thought. Between 10% and 50% of the populations were anti-HEV-positive in various epidemiological studies [20–22]. Thus, millions of – usually clinically silent – infections occur each year in Europe but only a small proportion of infected individuals develop clinical symptoms. HEV infection should also be considered in the differential diagnosis of drug-induced liver disease [23,24], which has also a major importance in the management of liver transplant recipients, receiving various medication before and after transplantation. Of note, HEV has also been linked to extrahepatic manifestations and thus, the clinical implications

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Abbreviations: HEV, hepatitis E virus; HVR, hypervariable region; ORF, open-reading frame; GBS, Guillain–Barrè syndrome.

of hepatitis E virus infections are likely to be much broader than previously estimated [25–28].

Key Points

- Hepatitis E virus infection can take chronic courses in immunocompromised individuals. There is limited data suggesting that chronic courses may be more frequent after liver transplantation
- Chronic hepatitis E is often associated with rapid progression to liver cirrhosis
- HEV genotype 3 infection can be a zoonosis and HEV is believed to be transmitted mainly by ingestion of raw uncooked meat. Other sources of transmission are food hygiene and transfusion of HEV positive blood products
- Different immunosuppressive drugs may have distinct effects on HEV replication (either inhibitory or stimulatory) and should therefore be selected accordingly
- Ribavirin can be used to treat HEV infection and should be given for at least 3 months. Treatment failures may occur and require further investigation

HEV virology

HEV is a small (27–34 mm) non-enveloped virus which belongs to the family of *Hepeviridae*. It is encoded by a single-stranded RNA (7.2 kb) which consists of three open-reading frames (ORF1–3) [29]. The non-structural proteins are encoded by ORF1 and their translation leads to the synthesis of proteins which are primarily essential for viral replication. In particular, it consists of a methyltransferase, a protease, a macrodomain, a helicase and an RNA-dependent RNA-polymerase [30]. Of note, a hypervariable region (HVR) is localized between the protease and the macrodomain. ORF 2 and ORF 3 on the other hand encode for the structural proteins. The capsid, which is essential for viral attachment and entry into liver cells, is the translational product of ORF2 [31,32]. ORF 3 partly overlaps with ORF2 and leads to the production of a small phosphoprotein, which is involved in the assembly and release of the virus and has been shown to interact with cellular host factors [33].

Similar to other RNA-viruses different genotypes of HEV exist due to a lack of proof-reading activity of the RNA-dependent RNA-polymerase. So far, 5 different HEV genotypes have been identified, which differ in their nucleotide sequences by 19%. Intra-genotypic differentiation into sub-genotypes is made upon a variation of approximately 12% [34]. These distinct genotypes differ markedly in their distribution, clinical presentation and species-specificity.

It is believed, that only genotypes 1, 2, 3 and 4 are able to cause apparent disease in humans, while genotype 5 has been identified only in birds thus far [14]. While genotype 1 and 2 solely infect humans, genotype 3 and 4 are zoonotic pathogens with likely major reservoirs in pigs [35–37], wild boars and deer [38–40]. Some data suggest that genotype 1 may also be able to infect pigs [41], which however needs to be confirmed by additional studies. The worldwide distribution of the different genotypes varies markedly. Genotype 1 and 2 infections have been reported mainly in Asia, India and North-Africa. Genotype 2 HEV has been also identified in Mexico [14,42]. Genotype 3 is present in Western countries as well as in Asia and North America while genotype 4 has been detected in Asian- as well as in European countries [14]. However, genotype 4 might also play a minor role in Europe as supported by recent data, demonstrating evidence for a genotype 4 infection in France [43].

Additionally, several related viral strains have been identified in a variety of species like bats [44], chicken [45], ferrets [46], rabbits [47], rats [48] and trout [49]. However, it is believed that these viruses are not able to infect humans.

Routes of HEV transmission

Distinct HEV genotypes differ in their route of transmission. Contaminated water is the main source for genotypes 1 and 2 infections. Large hepatitis E outbreaks have been described in various African and Asian countries including India, China, Somalia, South Sudan or Uganda [8–10,14]. In contrast, HEV genotype 3 and 4 usually cause sporadic infections, most likely due to consumption of contaminated food. HEV-RNA has been detected in a variety of food products in particular porcine livers and pig sausages in France, the US, the Netherlands and Germany [35,36,50,51]. Consumption of such food increases the likelihood for the development of HEV-infection [43,51–54]. In line with this, people who have close contact to swine and deer – farmers, slaughterhouse-workers, veterinarians – display a significant higher prevalence of HEV-infections compared to the general population [55,56]. Recent publications have delineated that HEV-RNA is even more widely distributed than previously thought, and has been found in various food-products like shellfish [57], mussels [58,59], green vegetables [60] as well as field-grown strawberries [61] (Fig. 1).

HEV transmission by blood products and organ transplantation

Organ transplant recipients frequently receive blood products, either prior to transplantation, during, or after transplantation. In most Western countries, blood products are tested for HCV-RNA and HIV-RNA by mini-pool testing and often even for HBV-DNA. However, blood products are not tested for HEV-RNA, even if transfused to individuals at risk. Several previous case reports suggested that HEV transmission by blood products is possible [14,62], which can obviously also occur after liver transplantation as demonstrated recently by Coilly *et al.* [63]. Clinically healthy European blood donors may carry HEV RNA as reported during the last 2 years for Scotland [64], Sweden [65], England [66], Germany [67,68] and the Netherlands [69] – even though the absolute HEV RNA prevalence is low (0.01–0.04%). Still, the risk for HEV transmission may become substantial if blood products are pooled. In this case, the risk for HEV transmission seems to be substantial as up to 10% of pooled plasma products tested HEV RNA positive in Europe [65]. Indeed, plasma products seem to be a specific cause for HEV infections [62,70]. However, screening of blood products for HEV has not been standardized in any country until now. Currently, there is an ongoing debate concerning this issue.

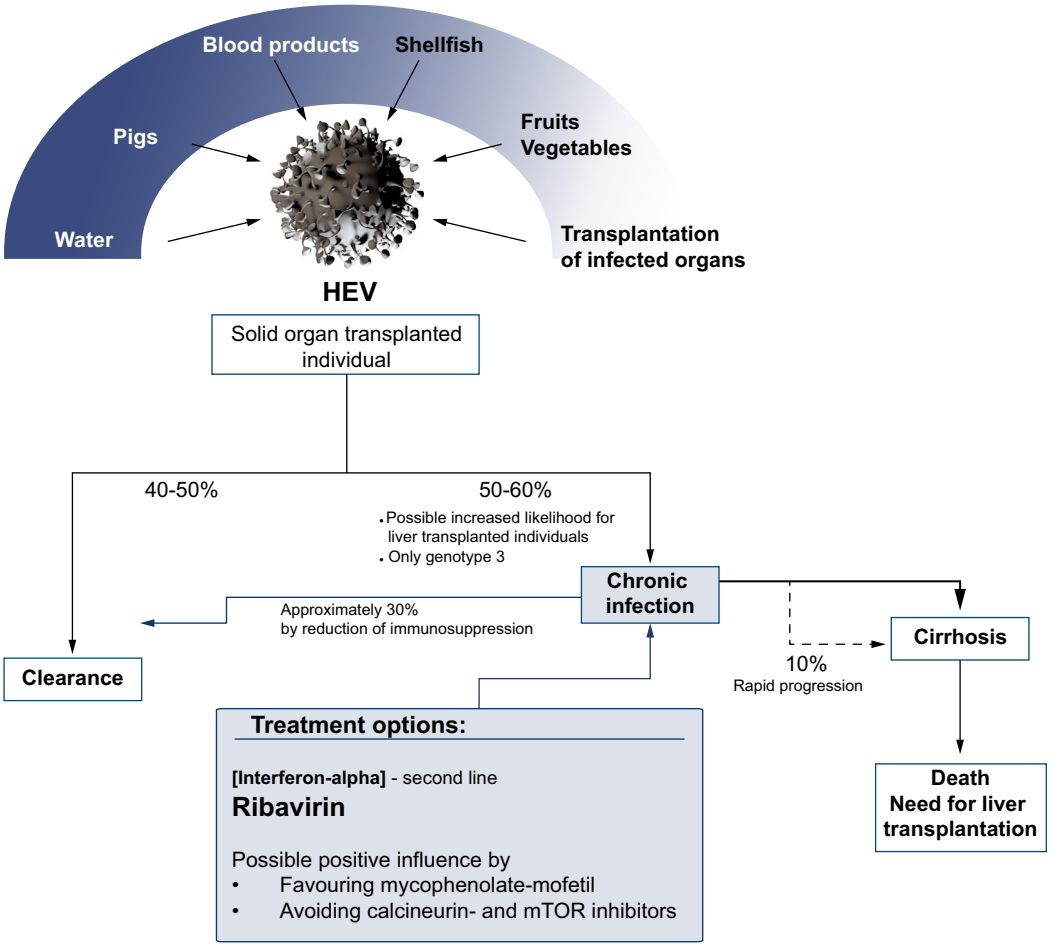

Fig. 1. Transmission and disease-progression in transplanted individuals. The possible mode of HEV-transmissions is illustrated. Additionally, known factors which influence the clinical course are depicted. Treatment options are highlighted in blue.

Recently, balancing miscellaneous aspects, Féray *et al.* delineated the potential significance of testing blood products for HEV [71]. This has been further supported by the work of Hewitt *et al.*, which was published in Lancet very recently. Here, the authors retrospectively screened blood donations that were collected in southeast England between October 2012 and September 2013 for HEV-RNA. Out of 225,000 blood donations, 79 patients were viraemic with genotype 3 HEV. The authors could follow-up 43 recipients of HEV-positive blood components and 42% of these individuals displayed evidence for infection with HEV thereafter. Short-term morbidity was rather low as only one of these individuals developed clinical significant symptoms. However, 10 patients, including organ transplant recipients who were receiving immunosuppressive medications, displayed prolonged viral infection [72].

HEV is not only transmitted by blood products but also by the transplanted organ itself (Fig. 1). Of interest, in one case report a HEV-RNA positive liver has been transplanted to a HEV-negative recipient. The development of a chronic HEV-infection rapidly led to re-cirrhosis of the graft [73]. It is important to note that organ transplants are not tested for HEV RNA. Stem cells may represent another risk for HEV infection. Recently, a stem cell donor has been identified who was clinically healthy at the time of evaluation but who suffered from acute hepatitis E at the time of leucopheresis [74].

Seroprevalence

The evaluation of the prevalence of HEV, as determined by the detection of anti-HEV IgG, is hampered by marked differences in the sensitivities and specificities of the antibody assays [75,76]. For example, a comparison of different commercial assays revealed a variation of anti-HEV IgG-positive individuals between 3.6% and 16.2% in blood donors from the UK [21]. The sensitivity thereby ranged from 56 to 98%. In the US an anti-HEV prevalence of around 25% has been proposed [77]. Similar variations can be seen in endemic areas where reported HEV prevalence may range between 30% and 80% [78]. It is well established that the HEV seroprevalence increases with age [20,22,79] but an overall decline of anti-HEV has been observed during the last two decades as e.g. recently shown for Germany. Here the authors screened 1092 sera and found a prevalence of 51% in 1996, which dropped to 34% in 2011 [22].

Clinical course of HEV infection

Clinical symptoms occur in only 2–5% of patients with acute HEV infection as suggested by data from a large, double-blinded placebo controlled vaccine trial from China where more than 110,000 individuals have been followed [80]. From apparent cases, mortality rates were estimated to be between 0.5–3% [81], leading up to 70,000 deaths worldwide each year [82]. Importantly, risk factors have been identified, which are associated with a more severe outcome of infection. In particular, pregnant women seem to be at risk where infection with HEV genotype 1 has been linked with foetal and maternal mortality in up to 25% of cases [3]. Distinct polymorphisms in the progesterone receptor may explain in part the particular severe outcome in pregnant women [79], but the detailed underlying pathomechanism has not been fully understood thus far. Fulminant disease progression seems also to occur more often in patients who already suffer from an underlying chronic liver disease [4–7]. Additionally, prolonged alcohol abuse serves as a risk factor for fulminant acute hepatitis E [83]. The course of acute HEV infection seems to differ between HEV genotypes as genotype 1 infection is associated with more severe cases than infection with genotype 3 HEV [84], however specific viral sequence variations could not be linked to fulminant hepatitis E [85].

Acute hepatitis E in immunocompetent individuals

The clinical course of acute hepatitis E in immunocompetent individuals is indistinguishable to infection with the hepatitis A virus. After an incubation period of 2–8 weeks initial symptoms are usually unspecific and include flu-like symptoms like myalgia, arthralgia, weakness, loss of appetite, abdominal pain and vomiting [86]. Together with the elevation of liver enzymes, in particular aminotransferases, alkaline phosphatase, γ-glutamyltransferase or bilirubin, liver-specific symptoms like jaundice, pruritus or decolouring of the stool can occur. These symptoms usually resolve spontaneously within 4–6 weeks [87]. Only a minority of infected individuals will develop fulminant hepatitis with the need for initiation of antiviral therapy and/or liver transplantation (Fig. 2A).

HEV infection in immunocompromised individuals

HEV infection may be associated with prolonged viraemia in immunocompromised individuals as shown for various cohorts of transplanted individuals [14]. Individuals may have impaired cellular and humoral responses against HEV, explaining the lack of control against HEV infection [14,88]. Similarly, HIV positive patients with low CD4+ cell count are at risk for chronic hepatitis E [15,16] as well as patients with other types of impaired cellular immunity [17–19]. In general, a viral infection lasting for 6 months or longer is considered a chronic infection. However, it has been suggested to classify HEV infection as chronic already after 3 months of persistent infection as no spontaneous clearance occurred thereafter in one study [89]. Overall, chronic courses of HEV infections have been reported in up to 50–60% of organ transplant recipients [90]. Chronic hepatitis E is frequently associated with a mild elevation of liver enzymes usually not exceeding 100–300 U/L and clinical signs of hepatitis are rare. However, fibrosis progression can be quite rapid within the first years of infection and several cases of fatal end-stage liver disease, possibly caused by chronic hepatitis E, have been reported [90–92]. It is important to note that chronic HEV infection has thus far been observed almost exclusively in genotype 3 infected individuals (Fig. 1 and 2). However, a recent report from China provided evidence, that genotype 4 HEV is also able to lead to a chronic course of infection as shown for a boy who received intensive chemotherapy due to an acute lymphoblastic leukaemia [93]. Interestingly, there was no evidence for chronic hepatitis E in a cohort of highly immunosuppressed kidney transplant recipients in India where HEV genotype 1 is the predominant genotype [94], indirectly suggesting that HEV genotype 1 may not cause chronic hepatitis E.

Extrahepatic manifestations

There is increasing evidence that HEV infection may also cause extrahepatic manifestations, supported by *in vitro* data which delineated that HEV is able to replicate in non-liver cells such as human intestine or monkey kidney cells [95]. In humans, HEV infection has been linked with a reduction in kidney function associated with persistent cryoglobulinemia [28]. Moreover, a variety of neurological symptoms may be explained in part by HEV infection [25–27]. A large case-control study from Rotterdam involving 201 patients with Guillain–Barrè syndrome (GBS) suggested that up to 5% of GBS patients had acute hepatitis E [27]. Whether these neurological symptoms are mediated by immunological features or are due to a direct infection of distinct brain cells with HEV is unclear. Of note, HEV RNA has been found in the spinal cord fluid of patients suffering from neurological disorders during HEV infection [96]. Interestingly, when the viral RNA genome was sequenced Kamar *et al.* found a phylogenetic distinct RNA arguing for an extrahepatic replication of the virus [97].

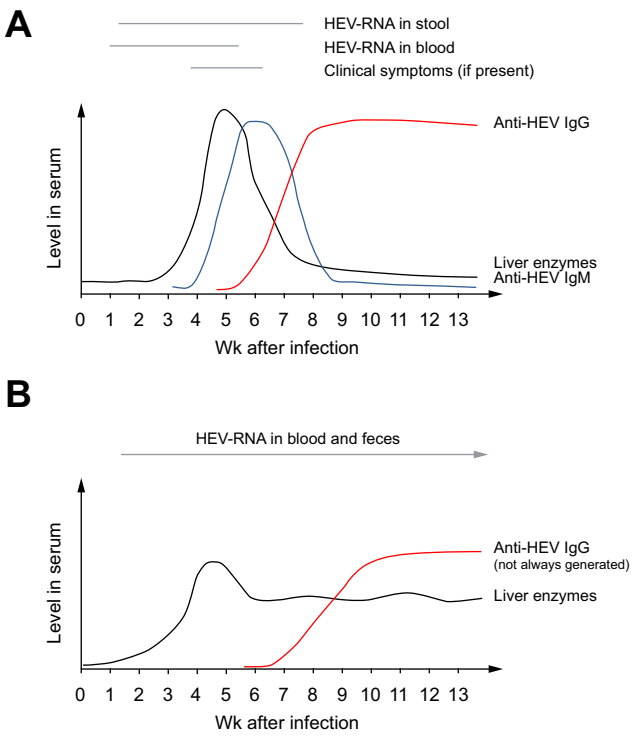

Fig. 2. Clinical signs and serological markers during HEV infection over time. (A) acute and (B) chronic HEV infection.

Further manifestations, associated with HEV include cases with acute pancreatitis, haematological abnormalities as well as myopathies [98]. Of note, extrahepatic symptoms may also develop even after HEV clearance as recently suggested for a liver transplant recipient who developed vasculitis and enteritis after HEV RNA became negative [99].

Diagnosis of HEV infection in immunosuppressed patients

HEV infection can be diagnosed in immunocompetent individuals by detection of anti-HEV IgM antibodies. Anti-HEV IgM titers increase with the onset of clinical symptoms. However, the analytical sensitivity of the anti-HEV IgM assays can differ [100], which potentially represents a major problem in patients receiving immunosuppressive medications. Indeed, delayed diagnosis of HEV infection has been reported in a liver transplant recipient due to variable performance of serologic assays [101]. Detection of anti-HEV IgG indicates successful clearance of a previous infection and may be associated with some level of protection against HEV re-infection even though HEV antibodies are not able to provide complete sterilizing immunity against HEV [102] (Fig. 2A). Anti-HEV-IgG can also diminish over time [103] and thus, seronegativity may not exclude previous exposure to HEV. Overall, testing for immunoglobulins has limitations in specificity and sensitivity [75,76,104]. Reliability seems more accurate in genotype 3 infection than in other genotypes. In addition, cross-reactivity to other viruses has been observed [105,106]. Anti-HEV testing may frequently be false negative in immunocompromised individuals. Time to seroconversion – meaning development of anti-HEV IgG – is delayed in patients after liver transplantation and some patients may never develop detectable anti-HEV IgG antibodies during chronic infection [92].

Considering the limitations of the antibody assays, diagnosis of HEV infection should be based on detection of HEV-RNA in organ transplant recipients. Still, even HEV PCR assays are not perfect as sensitivities may show substantial variability and most previous studies have been performed with in-house assays [107]. Moreover, not all published assays have been optimized for different HEV genotypes and a recent comparative study showed that up to one out of six HEV RNA positive samples were incorrectly classified as HEV RNA negative [108]. Importantly, the WHO established an internal control for RNA amplification technology in 2011, which should help to solve some of the technical issues [107].

An alternative to HEV RNA assays could be HEV antigen testing. HEV antigens may show different kinetics than HEV RNA and have been shown to be longer positive than HEV RNA in patients with acute hepatitis E [109]. A commercial sandwich ELISA for the detection of the HEV-capsid is available. However, this test seems to be less sensitive than RNA-techniques and the role for detection of infection needs to be evaluated in more detail in future studies [110] (Fig. 2B).

Prevalence and clinical course of hepatitis E in liver-transplant recipients (summarized in Table 1)

The first case series demonstrating chronic HEV infection in transplanted individuals also included three liver transplant recipients [13]. Overall, 14 solid-organ transplanted patients with acute HEV infections were identified. Out of these, 8 patients developed persistent infection, which interestingly included all three liver transplanted individuals. The study demonstrated a rather rapid histological disease progression within a median time of follow-up of only 18 months. Subsequently, various further studies have been published, addressing the role chronic infection in organ transplant recipients including liver-transplanted individuals. Overall, several cases of chronic hepatitis E after liver transplantation were identified even though the absolute frequency was low with 1–3% in most studies. Considerable differences between countries were evident.

In 2009, a study from the Netherlands was published [111], screening 285 patients, which had undergone liver transplantation, for HEV-RNA, anti-HEV IgM and IgG levels. 96% of these individuals were HEV RNA and anti-HEV IgG/IgM negative and thus, had no evidence for post-transplant HEV infection. One female patient was identified with ongoing chronic hepatitis caused by HEV – without displaying any HEV-specific antibody-titres. She subsequently had to be re-transplanted due to liver cirrhosis. However, the new graft became re-infected and persistent HEV infection sustained in the absence of anti-HEV antibodies. Overall, 9 patients were IgG positive after transplantation (3.1%). Retrospective analysis of stored samples revealed the presence of anti-HEV already before transplantation in 6 of these 9 individuals. Two patients seroconverted to anti–HEV IgG positive after transplantation. One of the patients developed anti-HEV IgG during a chronic phase of infection, which started 18 month after transplantation and lasted until 60 month post-transplantation. This patient then cleared the infection spontaneously. Whether a change in immunosuppressive medication was associated with HEV clearance was not reported in the publication.

The findings were later confirmed by another study from The Netherlands [112]. The authors retrospectively screened a large cohort of solid-organ recipients (total N = 1200, liver: n = 300) for HEV-RNA. In total, 12 patients were HEV-RNA positive of which 4 had undergone liver transplantation. One of these cleared the virus within a few days, all others – and in particular all of the liver transplanted – became chronically infected. Importantly, the authors also delineated that the time of initial HEV-infection and the first detection of anti-HEV-IgG spanned on average 32–124 days, supporting the need for evaluation of HEV-RNA when HEV infection is suspected in immunocompromised individuals.

The experience from the Netherlands was largely in line with observations from northern Germany, which were published in 2010 [92]. A retrospective screening of liver transplanted individuals tested 226 patients who all had elevated liver enzymes. HEV infection was identified as a probable cause of hepatitis in three individuals with two patients becoming chronically infected. One of the persistently infected patients developed rapid progression of liver fibrosis within 2 years. The prevalence of anti-HEV was higher in the liver transplanted group (4.4%) compared to 3% in non-transplanted individuals with chronic liver diseases and 1% in healthy controls.

Several studies from France investigated the prevalence of hepatitis E in immunosuppressed patients. Markers of HEV infection were studied in a cohort of liver transplanted individuals who received transplantation between 2005 and 2012 in the Rhône-Alpes-region [113]. HEV seroconversion was observed in 7.7% of patients after a median follow-up of 33 months. The authors estimated the HEV incidence rate to be 2.83 cases per

Table 1. Overview and hallmark studies investigating HEV infection in cohorts of liver transplanted individuals.

Author, [Ref.]	Year	Country/ area	Cohort	Seroprevalence/incidence	Hallmark of the study
Kamar *et al.,* New England Journal of Medicine [13]	2008	France	217 solid organ transplantation patients with elevated liver enzymes (liver: n = 86) (adult), retrospectively	prevalence 10%	14 solid organ transplanted individuals (3x liver, 9x kidney, 2x kidney and pancreas) had acute HEV infection, all liver donors developed chronic HEV infection; Metavir fibrosis score (in liver transplanted individuals) was assessed in two patients, one progressed in fibrosis (F1 to F2, Metavir activity score 0 to 3) in 15 month of follow-up, the other one did not show progression in 17 month
Haagsma *et al.,* Liver Transplantation [111]	2009	The Netherlands	285 liver transplanted patients (adult), retrospectively	prevalence 3%	One chronically infected individual cleared the virus spontaneously, 1 patient had to undergo re-transplantation due to chronic infection
Buti *et al.,* Liver Transplantation [115]	2009	Spain	108 solid organ recipient (kidney: n = 21, liver: n = 82, combined n = 5) (adult), retrospectively	prevalence overall 2.7%, in liver transplanted 3.7%	No patient with ongoing HEV-infection (drawback: only IgG-positive individuals were tested for IgM and HEV-RNA)
Halac *et al.,* Gut [116]	2010	Canada	80 liver transplanted individuals (children), retrospectively	prevalence 13-23%	2/3 of the patients which had signs of hepatitis post-transplant acquired anti-IgG-positivity over time. One individual had chronic infection and displayed cirrhosis of the graft
Pischke *et al.,* Liver Transplantation [92]	2010	Germany	226 liver transplanted individuals (adult), retrospectively	prevalence 4.4%	3 acutely HEV-infected patients, two became chronically infected; prevalence in liver-transplanted individuals 4.4% (n = 226), in non-transplanted individuals with liver disease 3% (n = 129) and 1% in healthy control (n = 108)
Legrand-Abravanel *et al.,* Emerging Infectious Diseases [114]	2011	France	871 solid organ transplanted patients (kidney: n = 700, liver: n = 171) (adult), retrospectively	prevalence overall 14%, liver transplanted 12%; incidence overall 3.2 per 100 person-years, for liver 4.8 cases per 100 person-years	6 liver transplanted patients became chronically infected; low age (under 52) and liver transplantation served as risk factors for acquiring HEV-infection
Kamar *et al.,* Gastroenterology [90]	2011	Multicenter study, Europe and US	85 solid organ transplanted patients with acute HEV-infection (liver: n = 24) (adult), restrospectively	no prevalence tested	Reduction of immunosuppression leads to clearance in 30% of the cases; liver transplantation as well as use of tacrolimus are risk factors for chronic infection, 66% of acutely infected, transplanted patients developed chronic infection; 14.3% of chronically infected patients developed liver cirrhosis. Two liver transplant patients required a second liver transplant due to HEV-related cirrhosis
Pas *et al.,* Emerging Infectious Diseases [112]	2012	The Netherlands	1200 solid organ recipients (heart: n = 259, lung: n = 53, liver: n = 300, kidney: n = 574, multiple: n = 14) (adult), retrospectively	no prevalence tested	12 patients were positive for HEV-RNA (liver: n = 4); 11 had chronic infection (liver: n = 4)
Buffaz *et al.,* European Journal of Clinical Microbiology & Infectious Diseases [113]	2014	France	206 liver transplanted individuals (adult and pediatric), retrospectively	prevalence 28%; incidence 2.83 cases per 100 person-years	3 chronically infected patients; 2 of these displayed anti-HEV IgG titers pre-transplantation which did not lead to protection of infection post-transplantation; seroconversion after liver transplantation was observed in 11 individuals during follow-up (mean 33 month)
Sherman *et al.,* Journal of Viral Hepatitis [139]	2014	USA	590 HIV-positive transplanted individuals (liver: n = 273)(adults), retrospectively	prevalence 18.9% in liver recipients	0.9-1.8% of liver transplanted individuals were anti-HEV IgM positive and none of the kidney transplant recipients; one patient with borderline anti-HEV IgM had cirrhosis; no patient was detected HEV-RNA positive
Galante *et al.,* ILTS, London [140]	2014	Germany	287 liver transplant recipients (adults), prospectively	no prevalence tested	4 patients had chronic HEV-infection

100 person-years. Only one of the seroconverted patients was also HEV RNA positive. In addition, the authors identified 2 patients with chronic hepatitis E who had already been anti-HEV IgG seropositive prior to transplantation. Based on this observation the authors concluded that HEV clearance before transplantation and presence of HEV antibodies may not protect from HEV infection in the context of immunosuppression after liver transplantation. However, one drawback of the study was that HEV-RNA-testing was performed only in individuals who were anti-HEV-IgM and IgG positive. Therefore, anti-HEV antibody-negative/HEV-RNA-positive patients would not have been detected with this screening approach.

In another study from Toulouse (France) a large number of kidney- (n = 529) and liver-transplanted (n = 171) individuals – transplanted between 2004 and 2008 – were retrospectively assessed for signs of HEV infection [114]. HEV infection after transplantation occurred in 34 patients (kidney recipients n = 22, liver n = 12). 14 patients seroconverted post-transplantation, while HEV-RNA remained negative. However, 20 patients who did not develop antibodies against HEV had detectable HEV RNA. The HEV incidence rates were calculated with 2.7 per 100 person-years in kidney recipients and 4.8 per 100 person-years in liver recipients. A chronic course of HEV infection was observed in 16 patients (47%). Strikingly, age under 52 at time of transplantation as well as receiving a liver transplantation were risk factors for acquiring HEV infection in the multivariate analysis.

There are also data from Spain. In 2010, Buti *et al.* investigated the HEV-prevalence in solid-organ-transplanted individuals by testing anti-HEV IgG when an elevation of liver enzymes was observed (kidney: n = 21, liver: n = 82, combined n = 5) [115]. Patients positive for IgG were also tested for anti-HEV-IgM as well as for HEV-RNA. Overall, only three liver transplanted patients were anti-HEV IgG positive. There was no evidence for persistent infections. Also in this study it has to be considered that HEV-RNA testing was performed only in anti-HEV IgG positive samples and thus the true prevalence of HEV infection could have been underestimated.

Factors potentially associated with chronic HEV infection in solid organ transplant recipients were investigated in a large multi-centre study involving 17 transplant centres throughout Europe and the US [90]. Overall 83 patients were enrolled including 26 liver transplant recipients. Interestingly, in this cohort the majority of liver-transplanted individuals developed chronic infection (22 of 26 infected individuals) which was significantly more frequent than in non-liver transplanted patients (34 of 59) supporting the hypothesis that the risk for persistent infections is higher after liver transplantation.

Sherman *et al.* studied HIV-infected kidney and liver transplanted individuals in the US for markers of HEV-infection. In 590 individuals (including 273 patients after liver transplantation) the authors found an anti-HEV IgG prevalence of 20% in both, liver and kidney transplanted patients. However, testing for HEV-RNA revealed no ongoing infection in any case.

The prevalence of HEV infection in children after liver transplantation was investigated in a Canadian study [116]. The authors screened 80 children transplanted between 1992 and 2010. Liver enzymes were normal in 66 children while 14 had persistently elevated ALT levels. Individuals with normal liver enzymes tested anti-HEV IgG positive in 15% of cases while two-thirds of the children with elevated liver enzymes seroconverted after liver transplantation. Additionally, seven of the children with elevated liver enzymes had detectable anti-HEV IgM more than once during follow-up and these patients also had histological signs of inflammation and fibrosis progression. One girl tested repeatedly HEV-RNA positive and unfortunately developed liver cirrhosis further highlighting the progressive nature of persistent HEV infection in immunosuppressed patients.

Role of immunosuppression in solid-organ transplanted individuals and HEV

Immunosuppressive drugs may interfere with replication of different viruses. It has, for example, been shown for HCV that steroids may increase HCV infectivity by upregulation of distinct entry receptors [117] and that cyclosporine can inhibit viral replication by interfering with cyclophilins [118]. There is also some evidence that the immunosuppressive regime may be important for HEV infection. Initial data suggested that chronic cases of hepatitis E are more likely in liver- and kidney transplanted patients if patients were treated with the calcineurin-inhibitor tacrolimus as compared to patients receiving cyclosporine [90]. In contrast, heart transplant recipients receiving mycophenolate did not develop persistent infection [91]. Of note, these clinical data have meanwhile been supported by studies investigating the effects of different drugs on HEV infection *in vitro*. The studies were possible due to the development of a robust *in vitro* model for HEV genotype 3 infection [119]. Interestingly, addition of tacrolimus increased viral replication supporting the clinical observations [120]. However, the other calcineurin inhibitor cyclosporine A also increased replication, which could not be confirmed yet in patient cohorts. Different mTOR-inhibitors, rapamycin and everolimus also led to an enhanced replication of HEV transfected cells *in vitro*, involving phosphorylated 4E-BP1 in infected hepatocytes [121]. However, the clinical impact of this finding is unclear at this stage. The use of corticosteroids does not seem to impact replication of HEV, both *in vivo* as well as *in vitro*. Strikingly, the use of mycophenolate-mofetil leads to a significant decrease of viral replication *in vitro*, which would on the first view support the above mentioned clinical observation in heart transplant recipients [91,120]. The mechanism is likely to be linked to IMPDH inhibition by mycophenolate [122]. However, mycophenolate-mofetil might also negatively influence leucocyte cell count and subsequently promote chronicity of infection [14,88]. Thus, it is unclear to what extent the beneficial *in vitro* effects of mycophenolate really translate into clinical benefits. Indeed, many patients with chronic hepatitis E in the literature received mycophenolate as part of their immunosuppressive regimen.

Overall, the choice of the immunosuppressive drugs seems to be of important for liver transplant recipients with hepatitis E. Still, both experimental and clinical data are very limited. At present one could suggest that tacrolimus and mTOR inhibitors should be avoided once chronic infection is evident, while steroids, cyclosporine and mycophenolate might be preferred compounds. However, there is not sufficient evidence yet to propose distinct "pre-emptive" immunosuppressive regimens to avoid chronic infections after exposure or to propose specific drug changes once persistent HEV infection has developed. Rather than discussing one drug *vs.* another it seems to be more important to reduce the overall level of immunosuppression as much as possible in patients with chronic hepatitis E.

HEV reactivation

In 2009 le Coutre *et al.* reported the case of a patient with acute lymphatic leukaemia who displayed an acute, self-limiting HEV infection prior to allogenic stem cell transplantation [123]. 14 weeks after transplantation reappearance of HEV viraemia was observed. Sequencing analysis confirmed the presence of identical viral strains prior to transplantation and at the time or reactivation suggesting prolonged viral persistence in the absence of detectable viraemia. For kidney-transplanted individuals no HEV reactivation has yet been described [124] while HEV can be transmitted by liver transplantation as for example delineated by Haagsma *et al.* [125] and Schlosser *et al.* [73].

Prevention of HEV infection

HEV, being a non-enveloped virus, is relatively robust and stable upon environmental harms. The risk of food-borne HEV can be significantly reduced by cooking of meat. *In vitro* assays suggest, that cooking meat for 1 min at 70 °C is efficient in reducing viral infectivity [126,127]. In theory, transmission by contact with raw meet seems possible, although not described in the literature so far. Therefore, this risk should be reduced by washing hands after exposure to potentially infected meat. Blood products are not routinely tested until now and it is unclear whether common inactivation procedures cause elimination of infectious HEV-particles. Although, direct human-to-human transmission of HEV is usually uncommon, patients should be advised to perform regular cleaning of shared sanitary facilities. If one presumes similar stabilities of HEV and HAV, HAV is partially resistant to 80% ethanol-based disinfectants, which should not be recommended alone for the decontamination of HEV either [128]. Overall, intra-family or direct person-to person transmission seems to be a seldom event but have been described and suggested in a few cases [129]. Therefore, testing of partners/family-members should be considered if risk factors for the development of severe infection are present including organ transplantation.

A vaccine against HEV was licensed in China which showed an efficacy of 94%-100% in a large-scale vaccine trial involving more than 100,000 individuals [80]. Unfortunately, this vaccine has not been approved yet in other countries and the efficacy to protect from HEV genotype 3 infections has also not formally been proven – even though the genotype 1- based vaccine was effective against HEV genotype 4 infections in China. A vaccine against HEV would be highly desirable, especially for patients at risk to develop fulminant acute HEV infection or to become chronically infected. However, even if a vaccine would be available it is unclear if the approach would be effective in immunosuppressed patients as antibody-titres might not be sufficient to protect against infection. This is supported by the study from Buffaz *et al.* who observed chronic HEV infections even in patients who displayed anti-HEV IgG before transplantation [113]. In line with this, Abravanel *et al.* recently also reported infection of solid-organ transplanted individuals, which were anti-HEV IgG positive prior to liver transplantation. They propose that an HEV antibody-titre below 7 WHO units/ml is not sufficient to protect from HEV-infection in this cohort. Additionally, they delineated that these infections can lead to both, acute and chronic HEV infection [130].

Treatment

HEV-infection is usually subclinical in immunocompetent individuals and does therefore not require specific therapies in the vast majority of cases. However, antiviral treatment of severe acute infections should be considered in patients with risk factors for fulminant liver failure. Ribavirin seems to be the treatment of choice as it can lead to a rapid decrease of viral load in patients with acute severe hepatitis E [131–133]. Of note, ribavirin was also beneficial not only in genotype 3 infection but also in one patient with very severe acute HEV genotype 1 infection [84]. Based on this – still rather limited – data, we would recommend to treat acute hepatitis E patients at risk of developing liver failure with ribavirin not only to prevent organ failure but also to avoid HEV transmission if liver transplantation is still needed. However, the specific role of ribavirin as pre-emptive therapy to avoid infection of the transplanted graft requires further investigation.

Chronic hepatitis E should be avoided in liver transplant recipients to prevent fibrosis progression. The first steps in the management of a transplanted patient with persistent HEV infection would be the re-evaluation of immunosuppressive drugs based on effects on HEV replication as discussed above and – if possible – to reduce the intensity of immunosuppression, which may lead to HEV RNA clearance in approximately 30% of cases [90]. If HEV is not cleared, antiviral therapies should be considered.

Two case reports describing in total 5 liver transplanted individuals showed that chronic hepatitis E may be treated with PEG-interferon alpha [134,135]. HEV RNA became negative over a treatment course of 3–12 months. However, interferon alpha treatment is associated with side effects and may also induce graft rejection. Thus, only well selected patients with chronic hepatitis E may be candidates of PEG-IFNα therapy.

First case series, reporting an antiviral efficacy of ribavirin monotherapy have been reported by two French centres in 2 and 6 individuals with persistent HEV infection, respectively [136,137]. Patients were treated for 3 months with a ribavirin dose of 600–800 mg per day and six of the eight patients experienced a virological response with sustained clearance of HEV, while two patients relapsed after the end of therapy. These findings were then reproduced by different centres. In Hannover (Germany), patients were treated for a longer time (5 months) and 9/11 patients achieved HEV RNA clearance. However and importantly, one patient showed a virological breakthrough when the ribavirin dose was reduced due to anaemia. Clinical resistance was evident as no further viral decline was observed even when the ribavirin dosing was increased again [84]. The so far largest cohort of ribavirin-treated patients was reported recently from France describing a retrospective analysis of 59 solid-organ transplanted patients with chronic hepatitis E [138]. Patients received ribavirin at a dose of 8 mg per kg body weight for a median of 3 months (range 1–18 months). A sustained virological response could be reached in 46 of the 59 patients. Interestingly, all patients who failed to clear HEV during the initial course of ribavirin therapy showed a sustained virological response when re-treated with ribavirin for a longer period. Overall, clearance of HEV was observed in 95% of the cases (2 patient still received retreatment, 2 patients died of HEV- and treatment-unrelated reasons, 2 patients declined to undergo re-therapy and 2 were lost during follow-up). In the multivariate analysis a low lymphocyte count was predictive for non-response

Table 2. Overview and hallmark reports investigating different treatment options of chronic HEV infection in liver transplanted individuals.

Author, [Ref.]	Treated individuals	Antiviral drug/intervention	Outcome/Hallmarks
Kamar *et al.,* 2010; Clinical Infectious Disease [92]	3 liver transplanted	PegIFNα-2a	2 patients cleared infection, 1 individual relapsed
Haagsma *et al.,* 2010; Liver Transplantation [135]	2 liver transplanted	PegIFNα-2a	One patient cleared under treatment; the other patient was treated for 16 weeks, but still displayed detectable HEV-RNA. This person resolved 4 weeks after discontinuation, during which period levels of tacrolimus were rather low
Pischke *et al.,* 2013; Liver International [84]	5 kidney-, 4 heart-, 4 lung-, 2 liver transplanted	Ribavirin or reduction of immunosuppression	Overall, reduction of immunosuppression led to HEV-clearance in three patients. 11 patients recieved ribavirin, 9 of these cleared the virus, one patient died due to treatment- and HEV-unrelated disease, one patient remained HEV-RNA positive and died later due to liver related disorders. Both liver transplanted individuals resolved by reduction of immunosuppression
Junge *et al.,* 2013; Pediatric Transplantation [141]	1 liver transplanted	Ribavirin	The patient cleared infection under treatment
Kamar *et al.,* 2014; New England Journal of Medicine [138]	37 kidney-, 5 heart-, 5 kidney & pancreas-, 2 lung-, 10 liver transplanted	Ribavirin	Overall, HEV clearance under therapy was achieved in 95% of the patients, recurrence of HEV-RNA was detected in 10 patients after the end of treatment, 7 of these patients underwent re-treatment which led to sustained virological response in 4 individuals, 2 were HEV-RNA negative and still recieved therapy and 1 patient was HEV-RNA negative 3 month after the end of re-treatment; in liver transplanted individuals 8 had sustained virological response, while 2 had no sustained virological response after initial treatment; a higher lymphocyte count at the initiation of ribavirin was significantly associated with a sustained virologic response; a positive test for HEV RNA at month 1 of treatment increased the likelihood for failure to achieve a sustained virological response
Klein *et al.,* 2014; Experimental and Clinical Transplantation [142]	1 liver transplanted	Ribavirin	The patient cleared infection under treatment
Galante *et al.,* 2014; oral presentation ILTS London[140]	4 liver transplanted	Ribavirin	3 patients resolved, 1 patient relapsed after therapy and is still receiving retreatment

to treatment. Of note, anaemia was a dominant side effect requiring reduction of ribavirin in 29% of the patients. This underlines that patients with poor performance status and comorbidities may not be applicable for ribavirin treatment and that alternative antiviral treatment are still needed.

Studies, investigating antiviral regimes specifically in liver transplanted individuals are not yet available. However, available data prompt for ribavirin being the treatment of choice (Table 2).

Concluding remarks

It is now well established that HEV infections can take chronic courses in immunocompromised individuals and that the risk for chronicity – even though based on limited data – may be even higher for patients after liver transplantation. However, the overall frequency of HEV infection in European and North American transplant cohorts is low. HEV RNA testing of patients with elevated liver enzymes only seems to be a reasonable approach. HEV genotype 3 infection is a zoonosis and liver transplant recipients have to be advised to avoid the consumption of raw and uncooked meat. Transfusion of HEV by blood has to be considered and HEV RNA testing of blood products is currently under debate in several countries. Chronic hepatitis E is often associated with a particular rapid progression to liver cirrhosis and should therefore not be missed. The choice of immunosuppressive drugs is important in case of HEV infections as distinct effects on HEV replication (either inhibitory or stimulatory) have been described. An antiviral treatment option is available with ribavirin, which should be given for at least 3 months, however, treatment failures may occur supporting ongoing research to identify additional treatment options for a frequent and largely underestimated infectious complication after liver transplantation.

Conflict of interest

Prof. Manns has received payments for consulting services from Abbott, Falk Foundation, Merck, and Roche.

Prof. Wedemeyer has received payments for consulting services from Abbott, Falk Foundation, Merck, Roche, Roche Diagnostics, and Siemens, as well as payments for his expert advice from Abbott, Roche, Roche Diagnostics, and Siemens.

Steinmann, PD PhD and Behrendt, MD have nothing to declare.

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